GENETIC CONTROL OF THE IMMUNE RESPONSE TO STAPHYLOCOCCAL NUCLEASE

III. Time-Course and Correlation between the Response to Native Nuclease and the Response to its Polypeptide Fragments

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Genes linked to the *H-2* major histocompatibility complex and controlling the immune response to a variety of macromolecular antigens have been described in mice (1). However, the genetic control of the immune response to only a few natural globular proteins has been studied (1–3) largely because of their antigenic complexity. Among these, staphylococcal nuclease (nuclease) is a convenient model, since it is a relatively simple protein, of mol wt only 16,800, consisting of a single polypeptide chain with no disulfide bridges. Its amino acid sequence and three-dimensional structure at high resolution have been determined (4, 5).

In a previous study, Lozner et al. (3) demonstrated that the antibody response of mice measured 3 wk after a single immunization with nuclease in complete Freund's adjuvant was under *H-2*-linked *Ir* gene control. The *H-2* linkage was demonstrated both by a comparison of congenic resistant strains, which differ only at *H-2*, and by a formal genetic linkage analysis in an F_2 generation. Furthermore, the *Ir* gene (referred to as "*Ir*-Nase") was mapped in the *I-B* subregion of *H-2* using recombinant mice.

We now present an analysis of the time-course of response to multiple immunizations with nuclease in four strains of mice. In addition we have analyzed the antibody response to several large polypeptide fragments from nuclease in five strains. Our results demonstrate that the magnitude of response to nuclease is controlled by additional non-*H-2*-linked genetic factors. Further, we have identified a second *H-2*-linked *Ir* gene (or genes) involved in the response to this globular protein. Lastly, we present evidence that the antibody response to one fragment of nuclease, comprising residues 99 to 149, appears to be under the same genetic control as the response to the native protein.

Materials and Methods

Mice. All mice were obtained from The Jackson Laboratory, Bar Harbor, Maine, and were 6–8-wk old at the start of each experiment. Only female mice were used except for the DBA/1 mice immunized with fragments (1–126) and (6–48), which were male. The strains used, with their *H-2* haplotypes and responder status from the earlier studies of initial response to whole nuclease,

were A/J ($H\text{-}2^a$, high), C57BL/10 Sn ($H\text{-}2^b$, low), B10.A/SgSn ($H\text{-}2^a$, high), SJL/J ($H\text{-}2^s$, high), and DBA/1J ($H\text{-}2^q$, low).

Immunization Schedule. 8–12 mice of each strain were used for each immunogen. Mice were bled from the tail, and the sera were stored frozen to be assayed individually. Preimmune serum was obtained from each mouse to be used as a control for that single mouse. Animals were immunized intraperitoneally with 6 nmol of antigen, native protein or fragment (equivalent to 100 μg of whole nuclease), emulsified 1:1 in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich., H37Ra). The first bleed was taken 21 days later, and the animals were then boosted with one-tenth as much antigen i.p. in 0.1 ml of phosphate-buffered saline (PBS).[1] The second bleed was obtained 10 days later, and successive boosts and bleeds were carried out at 10-day intervals.

Purification of Staphylococcal Nuclease. Nuclease was purified from the supernatant culture medium of *Staphylococcus aureus*, Foggi strain, by the method of Bohnert and Taniuchi (6). Additional purification steps were taken as described in the accompanying paper (7), to ensure the purity of the preparation.

Preparation of Fragments of Nuclease. Fragment (99–149). Fragment (99–149) was prepared by cyanogen bromide cleavage according to the method of Taniuchi and Anfinsen (8). The resulting product was judged over 90% pure by two-dimensional tryptic peptide mapping (9) and its ability to complement with fragment (1–126) to produce enzymic activity (4).

Fragment (1–126). Fragment (1–126) was prepared by the method of Taniuchi and Anfinsen (10). Fragment (127–149) was obtained as a byproduct, which was further purified by gel filtration on Sephadex G-50SF. The preparation of fragment (1–126) had less than 0.5% contamination with native nuclease after two passes through deoxythymidine diphosphate-Sepharose (pdTp-Sepharose) affinity columns as indicated by its sp act of 12 U/mg. It showed a single line on Ouchterlony double immunodiffusion plates against both goat antinuclease and goat anti-fragment (1–126).

Fragment (6–48). Fragment (6–48) was prepared by the method of Taniuchi et al. (11) and was a gift of Dr. John Gerlt. It was further purified by affinity chromatography on pdTp-Sepharose to remove any whole nuclease or fragment (49–149). The resulting purified fragment (6–48) had no detectable nuclease activity in a solution of 3.2 mg/ml of peptide.

Radiolabeling of Peptide Fragments. Fragments (99–149) and (1–126) were carbamoylated with [^{14}C]KCNO (Amersham/Searle Corp., Arlington Heights, Ill., specific radioactivity, 55 mCi/mmol) by the method of Stark (12) which leads to the selective carbamoylation of the amino-terminal α-amino groups 100-fold faster than the ε-amino groups of lysine residues. The procedure was modified as follows: for 0.81 μmol of fragment (99–149), a 70-fold excess of [^{14}C]KCNO was used at a final concentration of 0.2 M in 0.1 M triethanolamine hydrochloride pH 7.0, with a reaction time of 70 min at 25°C, followed by gel filtration on a 1 × 95-cm column of Sephadex G-10 in 0.1 M NH_4OAc, pH 7.4, to remove free cyanate. The resulting preparation had a specific radioactivity of 50 μCi/μmol corresponding to about 0.91 mol of cyanate incorporated per mol of peptide.

Fragment (1–126) was carbamoylated in a similar fashion except that a 50-fold molar excess of [^{14}C]KCNO was used and the reaction time was 90 min at 23°C. The final preparation had a specific radioactivity of 141 μCi/μmol corresponding to an average incorporation of 2.5 mol of cyanate per mol of peptide. 92% of the radioactivity was precipitable by 5% trichloroacetic acid in the presence or absence of excess unlabeled cyanate, and 91% could be precipitated by excess goat antinuclease with polyethylene glycol (see below).

Radioimmunoassay of Antibody Binding to Nuclease Peptide Fragments. Antibodies binding to ^{14}C-carbamoylated peptide fragments of nuclease were assayed by a modified Farr-type technique using polyethylene glycol to precipitate immunoglobulin and bound antigen based on the method of Desbuquois and Aurbach (13). 10 μl of the serum to be assayed was placed into the bottom of a 400 μl polyethylene conical microfuge tube (Beckman Instruments, Inc., Fullerton, Calif.), followed by 30 μl of PBS, pH 8.0, and 10 μl of an 80 μM solution of ^{14}C fragment (99–149) (25 μCi/μmol, about 44,000 cpm total) or 10 μl of a 70 μM solution of ^{14}C fragment (1–126) (29 μCi/μmol, about 40,000 cpm total) in PBS. After brief mixing on a Vortex-Genie mixer, the reaction mixture was incubated in capped tubes for 15–20 min at room temperature followed by 1–2 h at 4°C. Then 50 μl of prechilled 20% (wt:wt) polyethylene glycol in PBS, pH 8, (or 25% wt:wt in the case of

[1] *Abbreviations used in this paper:* PBS, phosphate-buffered saline (0.15 M NaCl, 0.015 M Na_2HPO_4, 0.004 M KH_2PO_4, pH 7.4); pdTp, deoxythymidine diphosphate.

fragment 99–149) was added. After vigorous mixing the tubes were centrifuged in a Beckman microfuge Model 152 at 10,000 *g* for 2 min at room temperature and then rechilled in ice. The clear supernatant solutions were transferred to 10 ml of Instabray (Yorktown Research Inc., Hackensack, N. J.) in a scintillation vial along with two rinses of 50 μl each of prechilled 10% (wt:wt) polyethylene glycol. These were counted on a Searle Mark III liquid scintillation counter for 10 min or 40,000 total counts to assess the fraction of free label. The pellets were dissolved in 100 μl of 1 N acetic acid (demonstrated not to quench) (or H_2O for fragment 99–149) and rinsed with 200 μl of H_2O followed by Instabray and counted as above to assess bound radioactivity. For assay of sera raised to fragment (6–48), 3 μl of 70 μM ^{14}C fragment (1–126) was used, and the vol of PBS was increased to 37 μl (see below).

The following controls were necessary in the development and interpretation of the assay: The optimum concentration and pH for polyethylene glycol giving quantitative precipitation of antibodies and bound antigen with the lowest blanks was 10–12% (wt:wt) at pH 8.0. The attainment of equilibrium was assured since no increase in binding occurred with incubation times from 1 min to 22 h, indicating rapid association kinetics. The antibodies were found to remain active in the polyethylene glycol until centrifuged to separate phases.

Blanks with control normal sera were usually close to 10% of the total radioactivity added, with no evidence for saturation even up to 10–20 μM free peptide. This observation suggests that the binding by normal sera is of low affinity ($K_A \leqslant 10^5\ M^{-1}$) and therefore probably "nonspecific." The antigen bound by the preimmune serum of each mouse was subtracted from that bound by each immune serum from the same mouse.

A preliminary binding curve on pooled serum was used to determine the concentration of antigen necessary to saturate the highest titered antiserum, and this constant concentration was used for all antisera to that particular antigen. Antisera to fragments (1–126) and (6–48) were assayed in duplicate.

Assay of Antinuclease Antibodies by Inhibition of Enzymatic Activity. The assay for antinuclease based on inhibition of enzymatic activity was modified from that described by Lozner et al. (3). 10 μl of a nuclease standard (5 μg/ml with sp act 2,000 U/ml) and 10 μl of a dilution of antiserum or saline control were incubated at room temperature for 5 min. An aliquot of 10 μl of this mixture was then transferred to 1 ml of nuclease assay mixture (3), and the change in absorbance at 260 nm for the experimental sample was subtracted from that for the control. The difference, after multiplication by the dilution factor, was defined as the units of nuclease activity inhibited per ml of serum, in which 1 U = 1 absorbance U/min, which corresponds to 32.8 pmol of nuclease at this specific activity. Although the assay depends on inhibition of enzymatic activity, at least in the case of goat antinuclease, no noninactivating antinuclease antibodies could be detected (D. H. Sachs, A. N. Schechter, A. Eastlake, and C. B. Anfinsen, unpublished observation).

It was noted that the apparent inhibition units per milliliter serum increased with increasing dilution of antiserum, but that usually a plateau was reached at high dilution. This nonlinearity was not observed when a goat antinuclease serum fractionated by affinity chromatography to be monospecific for the region 99–126 of nuclease was used (14). The most probable explanation for this nonlinearity thus seems to be that with multispecific antisera in which more than one antibody can bind simultaneously to a nuclease molecule, multiple hits are scored as single hits. This phenomenon is analogous to that which causes unusually steep displacement curves in radioimmunoassays (15). Therefore the amount of nuclease inhibited at high dilution of antiserum (large antigen excess), at which multiple hits are rare and the antibodies are saturated, is the best estimate of total antibody concentration. For this reason, multiple dilutions of antiserum were assayed, and the plateau value (when a plateau could be reached) or the highest value of inhibition units per milliliter serum was used.

Statistical Analysis. Mean of the differences between paired immune and preimmune sera was tested for significance by a two-sided Student's *t* test according to the method of Snedecor and Cochran for paired data (16). A two-way analysis of variance was performed (16) to compare the relative magnitudes of the experimental variance (judged by variation of replicates) and the variance due to actual variability among the 6–12 animals within a group.

A multivariate analysis of variance (17) using the program LDU 042 from the DCRT Program Library of the National Institutes of Health was performed to compare the significance of the differences of patterns of response of the five strains across the battery of the three antigens (and

three immune bleeds) and correspondingly to compare the three antigens across the panel of five strains.

Results

Progression of the Antibody Response to Nuclease with Boosting. The concentration of antinuclease in the serum of mice of the two highest responder strains, A/J and SJL, rose sharply with successive boosts of nuclease and reached a plateau after the third immunization (Fig. 1, left). The plateau level of response, about 1,000–1,400 inhibition U/ml of serum, corresponds approximately to 33–46 μM antibody binding site concentration.

In contrast, the antibody response of the B10.A mice (Fig. 1, right) increased only about threefold to a plateau level after four immunizations of about 120 inhibition U/ml of serum, only one-tenth the maximum response of the A/J mice. Since the A/J and B10.A strains share the same *H-2* haplotype, this marked difference throughout the course of the response must be attributed to one or more non-*H-2*-linked genes. Thus, within the group of strains classed as high responder in *H-2* type, there are additional non-*H-2*-linked factors which influence the magnitude of the antibody response to nuclease.

When the progression of antibody response with boosting was compared in the two strains bearing the same B10 genetic "background" (non-*H-2*) genes (Fig. 1, right), the B10.A ($H\text{-}2^a$, high responder) response was initially four times higher than that of the C57BL/10 ($H\text{-}2^b$, low responder) in agreement with previous observations (3). However, after three immunizations a difference was no longer observed. With further immunization, the C57BL/10 made just as much antinuclease as the B10.A. This phenomenon was reproducible in a second group of five C57BL/10 and nine B10.A mice that were given three immunizations (data not shown).

Antibody Responses to Nuclease Fragments. An artist's representation of the three-dimensional structure of nuclease is shown in Fig. 2. The region from residues 99–149 contains two of the three α-helices in the native protein. The region from residues 1–126 overlaps the former region from residues 99–126. A subregion of fragment (1–126), from residues 6–48, representing roughly the amino-terminal third of the whole protein, was also studied. Each of the three fragments was used as immunogen to prime and boost mice of the five strains studied.

The mouse antibodies to fragments (99–149) and (1–126) discussed below have been shown to be specific for the random conformation[2] in that they react with native nuclease about two orders of magnitude less well than with the random conformation fragments (data not shown).

Antibody Response to Nuclease Fragment (99–149). The mean concentration of antibody binding sites to fragment (99–149), measured as the difference in saturation binding between immune and preimmune serum from the same mouse, is shown in Fig. 3 for each of five strains. As in the response to whole nuclease, the antibody response to fragment (99–149) was found to be high in A/J and SJL, intermediate in B10.A, and low in C57BL/10 and DBA/1. However,

[2] "Random" conformation is used to mean the array of many non-native conformations in equilibrium with one another in solution.

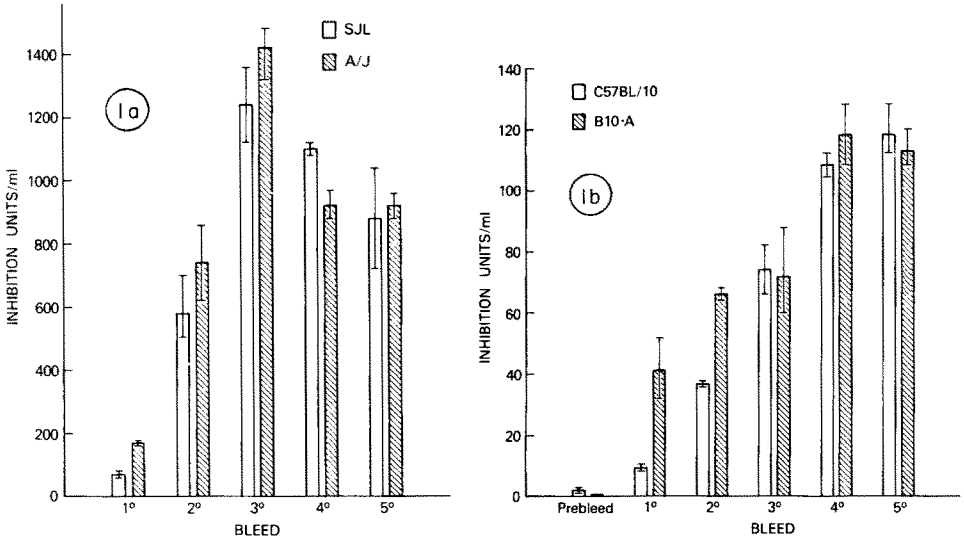

FIG. 1. Antibody response of four strains of mice to staphylococcal nuclease. (*a*) open bars, SJL/J; hatched bars, A/J. (*b*) open bars, C57BL/10; hatched bars, B10.A. Assay and inhibition units are as described in Materials and Methods. Numbers on the abscissa represent the number of immunizations before the bleed from which the sera are assayed. All data are from sera pooled from 8 to 12 animals. Error bars represent ranges of two to four replicates, not standard errors.

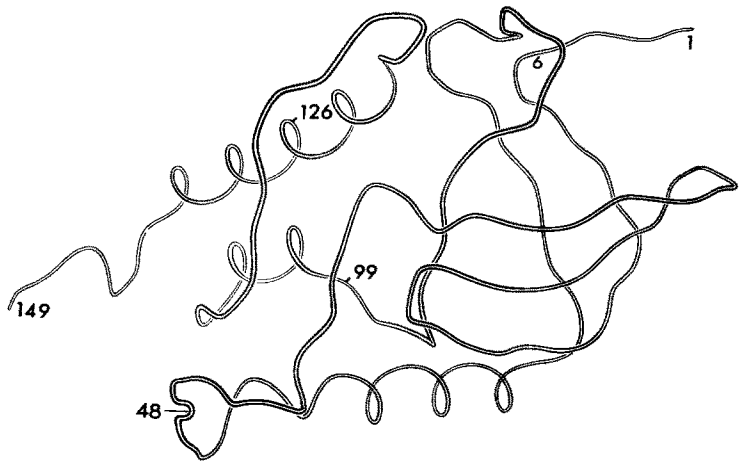

FIG. 2. An artist's representation of the three-dimensional structure of staphylococcal nuclease (8). Numbers indicate approximate positions of those residues demarcating the fragments under study: (6–48), (1–126), and (99–149).

whereas the antibody response to whole nuclease was low but significant in the initial sera of the C57BL/10 mice and increased with boosting, the response to fragment (99–149) was not statistically different from zero in this strain even after three immunizations. Thus, within the limits of resolution of the present assay system, responses to the fragment (99–149) were all-or-none rather than high or low.

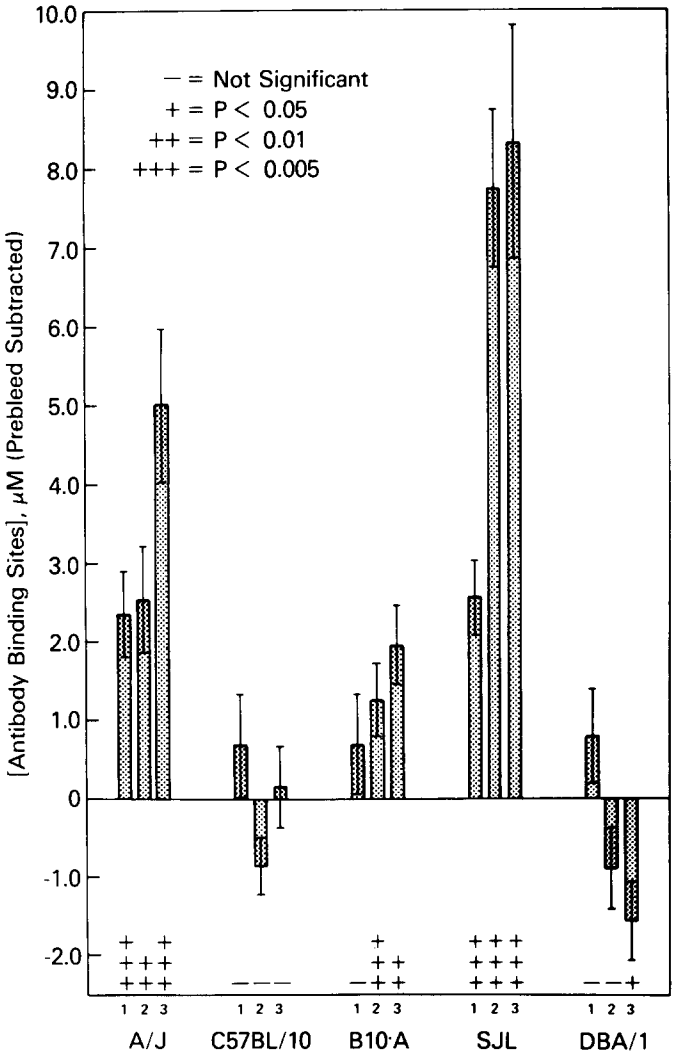

FIG. 3. Antibody response of five strains of mice to nuclease fragment (99–149). The strains of mice are indicated at the bottom. The numbers directly above the strain designations indicate the number of immunizations before the bleed shown. Response was measured as concentration of specific binding sites determined from the difference in binding at large antigen excess between the given immune bleed and the preimmune bleed from the same mouse, by radioimmunoassay (see Materials and Methods). The bars represent the means of these differences from preimmune bleeds for 5–12 individual mice in each group, and the error bars are standard errors of the mean. The + and − symbols above the bleed numbers indicate the level of statistical significance of the mean of the differences relative to zero, determined by a two-sided Student's t test, according to the key in the figure.

The mean binding by the DBA/1 sera after three immunizations was lower than the mean of the preimmune sera from the same mice, although the difference was of borderline statistical significance ($P < 0.05$). We are currently pursuing possible implications of this observation.

Antibody Response to Nuclease Fragment (1–126). The concentrations of

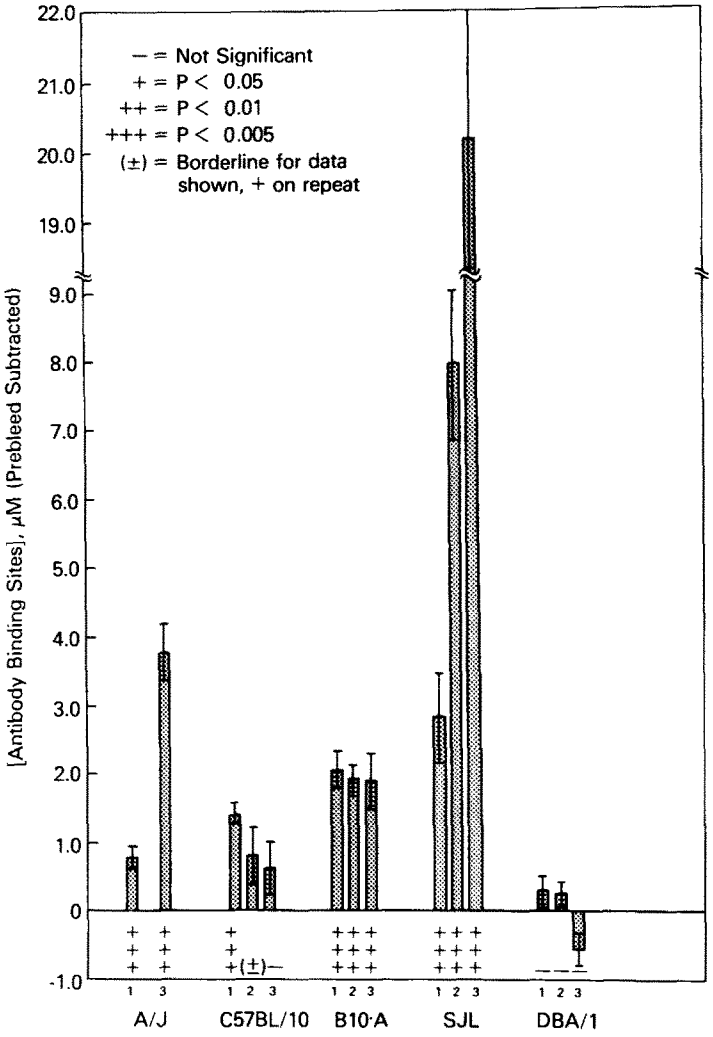

FIG. 4. Antibody response of five strains of mice to nuclease fragment (1-126). The experimental details and format of the figure are as in Fig. 3.

antibodies made to fragment (1-126) by mice immunized and boosted in the same fashion as with fragment (99-149) are shown in Fig. 4. A multivariate analysis of variance demonstrated that the difference in pattern of response between fragment (99-149) and fragment (1-126) across the panel of five strains was highly significant ($P < 4 \times 10^{-7}$). The major comparisons to be made with the response to fragment (99-149) and to whole nuclease are as follows: (*a*) The C57BL/10 mice showed a statistically significant response to this fragment in the first two bleeds, in contrast to their failure to respond to fragment (99-149). The initial response was, in fact, higher than that of the A/J mice and comparable to that of the B10.A mice. The apparent decrease in antibody level with boosting was not statistically significant by Student's *t* test and was not reproducible when the same sera were reassayed using 0.3 times as much antigen to

decrease background controls. (*b*) The response of the B10.A mice to fragment (1–126) was much closer to that of the A/J mice, in contrast to the responses to fragment (99–149) and to whole nuclease itself. (*c*) The SJL was a very high responder to fragment (1–126). Whereas both the initial and hyperimmune responses of SJL mice were comparable to those of the A/J mice for whole nuclease (Fig. 1) and for fragment (99–149), they were more than fivefold higher than those of the A/J mice for fragment (1–126). (*d*) The DBA/1 mice were nonresponders to both fragments as well as to whole nuclease.

Antibody Response to Nuclease Fragment (6–48). The antibody response to fragment (6–48) of nuclease was <0.3 μM in binding sites in all strains tested. While this fragment serves as a control (in comparison with fragment [99–149]), indicating that size and charge are not the sole determinants of responsiveness patterns, it is not very useful for comparing the pattern of response among the five strains because of this low immunogenicity. It is nevertheless interesting that the initial response of the C57BL/10 mice was significant and nearly as high in magnitude as the A/J response, although the later bleeds did not show statistically significant binding. In this sense, the pattern was more like that for fragment (1–126) (of which 6–48 is a part) than for fragment (99–149).

Statistical Analysis of Components of Variance. An analysis of the relative contributions to the standard errors (Figs. 3 and 4) of experimental error, as judged by variation among replicates, and of animal variability indicated that in most cases the experimental error was less than or comparable to the variance due to true biological variability among the animals within a group.

Discussion

The striking difference between the A/J and B10.A strains which share the same "high responder" $H\text{-}2^a$ haplotype indicates additional genetic factors in the control of the antibody response to nuclease that are not *H-2* linked. Polygenic control of the response to a complex antigen is not surprising. This non-*H-2*-linked genetic control may be similar to that observed by Dorf et al. (18) for the random terpolymer poly-(L-Glu, L-Ala, L-Tyr) in the same pair of genetic backgrounds, C57BL and A.

The ability of the "low responder" C57BL/10 after multiple immunizations to raise an overall response to nuclease which is equal to that of the congenic high responder B10.A is further indication of the complexity of the genetic control.[3] Therefore, we have pursued the response to fragments of the antigen in the current study and have examined the specificities of antibodies produced to native nuclease in the accompanying paper (7).

The patterns of antibody response to whole nuclease and three of its fragments for the five strains of mice tested are summarized in simplified form in Table I. Although this table oversimplifies some of the details apparent in Figs. 3 and 4, it facilitates the discernment of certain general patterns.

First, from a comparison of the vertical columns in the table, it appears that the response to fragment (99–149) best fits the original genetic distribution of the

[3] It is noteworthy that this masking of the *H-2*-linked effect with boosting is the opposite to that for the *H-2*-linked *Ir* gene controlling responsiveness to poly(Tyr,Glu)-poly(D,L-Ala)--poly Lys, in which the *H-2*-linked control is best detected after boosting (19).

TABLE I
Summary of Response Patterns to Whole Nuclease and Nuclease Fragments

Strain	Antigen			
	Whole nuclease	Fragment 99-149	Fragment 1-126	Fragment 6-48
A/J	High	High	High	Low
C57BL/10	Low	Non	Low to intermed	Low
B10.A	Intermed	Intermed	Intermed	Non
SJL	High	High	Very high	Low
DBA/1	Non	Non	Non	Non

Responder status is given as non for those with mean response not statistically different from zero, or as qualitative relative level of response (low, intermed, high, or very high responders) for those with mean response statistically significantly greater than zero (all relative to prebleeds from same mice).

whole antinuclease response in terms of the relative magnitudes of the responses among the five strains. In fact, whereas the C57BL/10 was a low responder to whole nuclease it appears to be a nonresponder to fragment (99–149). This observation suggests that the low response seen to whole nuclease in this strain may be due to a response to determinants between residues 1 and 99, and that this is manifested in a response to fragment (1–126) almost as high as that of the B10.A. This interpretation is in agreement with the results of a study of the T-cell proliferative response to nuclease and its fragments (R. H. Schwartz, J. A. Berzofsky, A. N. Schechter, and D. H. Sachs, manuscript in preparation). The specificities of the antibodies produced are examined in this regard in the accompanying paper (7).

This similarity of response pattern for whole nuclease and one of its fragments is only a correlation, and not proof that the same gene controls their respective responses. If the same gene is responsible, the *Ir*-nuclease gene defined earlier (3) may be involved in the control of recognition of a determinant in the region between residues 99 and 149. It is of conceptual interest that the *Ir*-gene control appears clearly all-or-none when one looks at a restricted region of the protein antigen rather than high-or-low as found for the whole complex antigen. This phenomenon may explain why most *Ir* genes have been defined for relatively simple antigens with few distinct determinants. The presence of other determinants which an animal can recognize partially masks selective defects in the immune response to a whole complex antigen.

Second, it is apparent from Table I that the DBA/1 mice are nonresponders to nuclease and any of its fragments, even after multiple boosts. Thus, if we compare the patterns of the DBA/1 and C57BL/10 mice, it would appear that the DBA/1 mice have at least one additional defect not found in the C57BL/10 mice. In this way we may tentatively define an additional *Ir* gene (or genes) involved in the antibody response to nuclease. Since in an earlier study (3) the SJL × DBA/1 combination was subjected to a formal genetic analysis in the F_2 generation which showed *Ir*-gene linkage to *H-2*, and since the SJL is a responder to all the regions of nuclease tested, it is probable that both *Ir*-gene defects in the DBA/1 strain are *H-2* linked. At the present time, however, it would be premature to conclude that both genes are in the *H-2* complex, since the F_2 animals were tested only for response to whole nuclease.

This definition of more than one *Ir* gene controlling the response to nuclease should not be confused with the two-gene hypotheses of Munro and Taussig (20) and Dorf et al. (21). Rather than being complementing genes which control different events in the response to the same antigen, the two or more genes suggested for nuclease are thought to control the response to different determinants on the molecule.

Third, we have noted that hyperimmunization of the nonresponder DBA/1 mice appeared on occasion to result in sera that have a lower binding activity for the immunogen than do the preimmunization sera from the same mice. If the observation represents a real immunological phenomenon, several possible explanations may be suggested, such as suppression of natural antibody or the production of anti-idiotype antibodies. Experiments are currently in progress to test these possibilities. However, the binding activity in the preimmune sera was of very low affinity ($<10^5$ M^{-1}) and is therefore not likely to be "natural" antibody.

Finally, the hypothesis that a fragment exhibiting predominantly "random" conformation[2] may be under the same *Ir*-gene control as the native protein from which it was derived suggests marked differences between the conformational specificity of the relevant receptors for *Ir*-gene control and of the antibodies ultimately produced. No ordered structure has been detected by physical means such as circular dichroism in either fragment (99–149) or fragment (1–126). In addition, the conformational equilibria of nuclease and its fragments have been studied immunochemically. Goat antibodies to the 99–126 region of native nuclease cross-react with fragment (99–149) with about 5,000-fold lower affinity (22). This difference in affinity has been attributed to a conformational equilibrium of the fragment such that only about one molecule in 10^3 or 10^4 is in a native-like conformation in solution at any moment (22). Analogously, goat antibodies to the random conformation fragment (99–149) cross-react with native nuclease with an affinity 2,900-fold lower than that for the fragment, a result suggesting a similar equilibrium for native nuclease, but in the opposite direction (23).

We have confirmed that the mouse antibodies made to fragments (99–149) and (1–126) cross-react very poorly with native nuclease. Thus, the antibodies ultimately produced distinguish strongly between native and random conformation. If it is not coincidental that the response to the random fragment (99–149) is under genetic control in the same pattern as that to native nuclease, two explanations may be suggested: either (*a*) the relevant determinant on this antigen which determines *Ir* gene-controlled recognition is either sufficiently flexible or sufficiently short that its predominant conformation in solution is the same in whole nuclease as in the fragment, or (*b*) the cell receptors which are relevant for *Ir* gene control of nuclease are much less conformationally specific than the antibody ultimately produced. At present, we cannot distinguish between these possibilities, nor do we know how generalizable this phenomenon may be to other systems.

Summary

The progression of the *Ir* gene-controlled antibody response to staphylococcal nuclease in mice with repeated immunizations has been examined. *H-2*-linked

control of the response to a single immunization with 100 μg of nuclease in complete Freund's adjuvant was confirmed. However, among strains of the high responder *H-2*^{a} haplotype, the response of the A/J mice was about 10-fold higher than that of the B10.A, indicating additional non-*H-2*-linked control. In addition, the low responder C57BL/10 (*H-2*^{b}) strain produced antibody levels as high as or higher than those of the congenic high responder B10.A (*H-2*^{a}) strain when both strains were repeatedly immunized, indicating complexity even in the *H-2*-linked control of the response to this small monomeric protein.

Polypeptide fragments of nuclease were also studied as immunogens. The antibody response to one fragment (residues 99–149) was found to follow the same pattern among five strains tested as that to whole nuclease. However, in this case the C57BL/10 was found to be a nonresponder rather than a low responder, failing to develop a response despite repeated immunizations. In contrast, the C57BL/10 showed a low but significant response to another fragment (residues 1–126) of nuclease. These results suggest that the apparent *H-2*-linked control of the response to whole nuclease is a reflection of the ability to recognize a determinant(s) in the region from residues 99 to 149, and that the eventual response of the C57BL/10 strain after hyperimmunization reflects the recognition of other determinants. If these observations reflect the common recognition of a determinant on native nuclease and on a random-conformation fragment, they have implications about the conformational specificity of the receptors, or the flexibility of the determinants, involved in *H-2*-linked *Ir*-gene control. In addition, evidence is presented for a possible second *H-2*-linked gene (or genes) controlling the response to other determinants of nuclease expressed on the polypeptide fragments.

We are grateful to Dr. Hiroshi Taniuchi for advice in the purification of nuclease and preparation of fragments, Mr. H. Robert Baird and Mrs. Karen D. Pettigrew for performing the multivariate statistical analysis of variance, Dr. John Gerlt for a gift of fragment (6–48), Mr. Walter Lyles for assistance in animal handling and care, and Doctors Christian B. Anfinsen, Benjamin Schwartz, Ronald H. Schwartz, and William E. Paul for helpful discussion, and Mrs. Dorothy Stewart for assistance in the preparation of the manuscript.

Received for publication 28 July 1976.

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